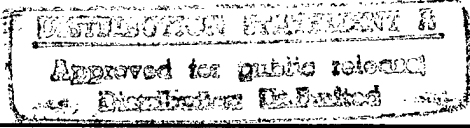


REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 7 Oct 1997	3. REPORT TYPE AND DATES COVERED Final 1 Jan 1996 - 31 Dec 1996		
4. TITLE AND SUBTITLE Mechanisms of viral infection in marine brown algae		5. FUNDING NUMBERS PN 97-PR-0097-00		
6. AUTHOR(S) Russel H. Meints				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Oregon State University Dept. of Botany & Plant Pathology Corvallis, OR 97331-2902		8. PERFORMING ORGANIZATION REPORT NUMBER N0019A		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research Dept. of the Navy		10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES		19971017 159		
12a. DISTRIBUTION / AVAILABILITY STATEMENT No limitations 		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) Eukaryotic marine algal viruses are large, dsDNA viruses. <i>Feldmannia</i> species Virus (resolved in two genome size classes 158 and 178 kbp) was developed as our prototype study systems. This virus infects marine brown algae. In nature sporophytic plants develop both plurilocular (mitotic) sporangia producing 2N spores and unilocular (meiotic) sporangia producing N spores. 2N spores normally yield adult sporophytes; haploid spores produce male and female gametophytes whose spores are the gametes for the sexual cycle. In the virus infected plant this life cycle is altered. Sporangia from virus-infected sporophytes do not produce spores. Instead unilocular sporangia contain virus particles. We show that the virus genomes exists in an integrated form within all other cells. All together the data suggest an integration/excision mechanism that employs an integrase/recombinase and conservative site-specific recombination. This enzyme complex appears to include topoisomerase-like activities which recognize sites within the virus and host. Unlike previously described systems we expect blunt end cutting and ligation or single bp. A large family of 173 bp repeat elements in the FsV genome was characterized. Two ORFs for "RING" zinc finger bearing genes were found as were two protein kinase genes. Northern blots demonstrated 6 major and 18 minor transcripts. The most abundant transcript was the major structural protein. Sequence analysis indicated significant homology with proteins of <i>Chlorella</i> -virus, Iridioviruses and African Swine Virus.				
14. SUBJECT TERMS Brown algae viruses		15. NUMBER OF PAGES		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT UL	

FINAL REPORT

GRANT #: N00014-93-1-0251

Project No. 97-PR-0097-00

PRINCIPAL INVESTIGATOR: Dr. Russel H. Meints

INSTITUTION: Oregon State University

GRANT TITLE: Mechanisms of Viral Infection in Marine Brown Algae

REPORTING PERIOD: 1 January 1996-31 December 1996

AWARD PERIOD: 1 January 1993 - 31 December 1996

OBJECTIVE: To carry out the first molecular characterization of a virus infecting a eukaryotic marine alga, and to determine whether this alga-virus system can be used as an experimental genetic tool for the study of the molecular genetics of brown algae (Phaeophyceae). To determine the insertion sites of the virus in the host genome and determine if this information could be exploited to develop a transformation system.

APPROACH: Virus-infected *Feldmannia* is maintained in laboratory culture. Viruses are purified from the cultures for study of virion structure and viral genome characteristics. The viral genome was cloned in a cosmid library to permit mapping of its structure, as source material and for probe analysis, and the nucleotide sequence of genes with abundant transcripts were determined to compare with known sequences and to provide promoter regions for construction of vectors for use in transformation experiments. Cosmid probing of gametophyte host DNA was provided the possibility of obtaining the virus insertion sites.

ACCOMPLISHMENTS (Final): Eukaryotic marine algal viruses are large, dsDNA viruses. *Feldmannia* species Virus (resolved in two genome size classes 158 and 178 kbp) was developed as our prototype study systems. This virus infects marine brown algae. In nature sporophytic plants develop both plurilocular (mitotic) sporangia producing 2N spores and unilocular (meiotic) sporangia producing N spores. 2N spores normally yield adult sporophytes; haploid spores produce male and female gametophytes whose spores are the gametes for the sexual cycle. In the virus infected plant this life cycle is altered. Sporangia from virus-infected sporophytes do not produce spores. Instead unilocular sporangia contain virus particles. We show that the virus genomes exists in an integrated form within all other cells. All together the data suggest an integration/excision mechanism that employs an integrase/recombinase and conservative site-specific recombination. This enzyme complex appears to include topoisomerase-like activities which recognize sites within the virus and host. Unlike previously described systems we expect blunt end cutting and ligation or single bp. A large family of 173 bp repeat elements in the FsV genome was characterized. Two ORFs for "RING" zinc finger bearing genes were found as were two protein kinase genes. Northern blots demonstrated 6 major and 18 minor transcripts. The most abundant transcript was the major structural protein. Sequence analysis indicated significant homology with proteins of *Chlorella*-virus, Iridioviruses and African Swine Virus.

SIGNIFICANCE: This first molecular characterization of a marine algal virus, and first investigation of the host range of a marine algal virus should provide important basic information about these poorly known marine pathogens. Development of a genetic transformation system for marine algae would greatly enhance the potential of these organisms for production of biomass energy and biopolymers through biotechnology.

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Oregon State University; 1997

<u>The System</u> Filamentous brown alga Viruses described (158 & 178 kbp) Virus observed only in sporangium <u>Virus replication and expression</u>	<u>Objectives</u> Describe viral biology Obtain viral insertion sites Characterize viral fine structure <u>Develop mechanisms for transformation</u>
<u>Accomplishments</u> Completed map of two genome classes and subvariant of each Completed isolation and characterization of insertion site Completed transcript analysis and characterization of major structural protein, 2 "Ring" zinc finger proteins Completed isolation and characterization of DNA polymerase gene, 2 protein kinases	<u>Significance</u> First description of algal virus integration site First description of marine algal virus major capsid protein Progress toward development of transformation system